Applicant: Walter Callen et al.

Serial No.: 09/656,309

Filed

: September 6, 2000

Page

: 3 of 19

Amendment to the Claims:

Please amend the claims as follows.

This listing of claims will replace all prior versions, and listing, of claims in the application:

Attorney's Docket No.: '09010-027003 / DIVER1350-2

Listing of Claims:

Claims 1 to 30 (canceled)

Claim 31 (currently amended) A method of generating a <u>nucleic acid encoding</u> variant that encodes a polypeptide having <u>a</u> polymerase activity comprising:

obtaining a nucleic acid comprising a sequence having at least 70% identity to the sequence set forth in SEQ ID NO:1 and encoding a polypeptide having <u>a</u> polymerase activity, or its complement; and

modifying one or more nucleotides in said sequence to another nucleotide, deleting one or more nucleotides in said sequence, or adding one or more nucleotides to said sequence, to generate a variant <u>nucleic acid</u> that encodes a polypeptide having polymerase activity; and

screening the polypeptide for a polymerase activity, thereby generating a polypeptide having a polymerase activity.

Claims 43 to 52 (canceled)

53. (currently amended) A method of generating a <u>nucleic acid encoding variant</u> that encodes a polypeptide having <u>a polymerase activity comprising</u>:

obtaining a nucleic acid comprising a sequence as set forth in SEQ ID NO:1, or its complement; and

modifying one or more nucleotides in said sequence to another nucleotide, deleting one or more nucleotides in said sequence, or adding one or more nucleotides to said sequence, to generate a variant <u>nucleic acid</u> that encodes a polypeptide having polymerase activity; <u>and</u>

CI

Applicant: Walter Callen et al. Attorney's Docket No.: 09010-027003 / DIVER1350-2

Serial No.: 09/656,309

Filed: September 6, 2000

Page : 4 of 19

screening the polypeptide for a polymerase activity, thereby generating a polypeptide having a polymerase activity.

54. (previously presented) The method of claim 53, wherein the modifications are introduced by a method selected from the group consisting of error-prone PCR, shuffling, oligonucleotide-directed mutagenesis, assembly PCR, sexual PCR mutagenesis, in vivo mutagenesis, cassette mutagenesis, recursive ensemble mutagenesis, exponential ensemble mutagenesis, site-specific mutagenesis, ligation reassembly, gene site saturated mutagenesis (GSSM) and any combination thereof.

- 55. (previously presented) The method of claim 53, wherein the modifications are introduced by error-prone PCR.
- 56. (previously presented) The method of claim 53, wherein the modifications are introduced by shuffling.
- 57. (previously presented) The method of claim 53, wherein the modifications are introduced by oligonucleotide-directed mutagenesis.
- 58. (previously presented) The method of claim 53, wherein the modifications are introduced by assembly PCR.
- 59. (previously presented) The method of claim 53, wherein the modifications are introduced by sexual PCR mutagenesis.
- 60. (previously presented) The method of claim 53, wherein the modifications are introduced by in vivo mutagenesis.
- 61. (previously presented) The method of claim 53, wherein the modifications are introduced by cassette mutagenesis.

Attorney's Docket No.: `09010-027003 / DIVER1350-2

Applicant: Walter Callen et al.

Serial No.: 09/656,309

Filed: September 6, 2000

Page : 5 of 19

62. (previously presented) The method of claim 53, wherein the modifications are introduced by recursive ensemble mutagenesis.

- 63. (previously presented) The method of claim 53, wherein the modifications are introduced by exponential ensemble mutagenesis.
- 64. (previously presented) The method of claim 53, wherein the modifications are introduced by site-specific mutagenesis.
- 65. (previously presented) A method of generating a <u>nucleic acid encoding</u> variant that encodes a polypeptide having <u>a</u> polymerase activity comprising:

obtaining a nucleic acid comprising a fragment of at least 30 consecutive nucleotides of a sequence having at least 70% identity to the sequence set forth in SEQ ID NO:1 and encoding a polypeptide having a polymerase activity, or its complement; and

modifying one or more nucleotides in said sequence to another nucleotide, deleting one or more nucleotides in said sequence, or adding one or more nucleotides to said sequence, to generate a variant <u>nucleic acid</u> that encodes a polypeptide having polymerase activity; and

screening the polypeptide for a polymerase activity, thereby generating a polypeptide having a polymerase activity.

66. (previously presented) The method of claim 65, wherein the modifications are introduced by a method selected from the group consisting of error-prone PCR, shuffling, oligonucleotide-directed mutagenesis, assembly PCR, sexual PCR mutagenesis, in vivo mutagenesis, cassette mutagenesis, recursive ensemble mutagenesis, exponential ensemble mutagenesis, site-specific mutagenesis, ligation reassembly, gene site saturated mutagenesis (GSSM) and any combination thereof.

Attorney's Docket No.: 09010-027003 / DIVER1350-2

Applicant: Walter Callen et al.

Serial No.: 09/656,309 Filed: September 6, 2000

Page : 6 of 19

67. (previously presented) The method of claim 65, wherein the modifications are introduced by error-prone PCR.

- 68. (previously presented) The method of claim 65, wherein the modifications are introduced by shuffling.
- 69. (previously presented) The method of claim 65, wherein the modifications are introduced by oligonucleotide-directed mutagenesis.
- 70. (previously presented) The method of claim 65, wherein the modifications are introduced by assembly PCR.
- 71. (previously presented) The method of claim 65, wherein the modifications are introduced by sexual PCR mutagenesis.
- 72. (previously presented) The method of claim 65, wherein the modifications are introduced by in vivo mutagenesis.
- 73. (previously presented) The method of claim 65, wherein the modifications are introduced by cassette mutagenesis.
- 74. (previously presented) The method of claim 65, wherein the modifications are introduced by recursive ensemble mutagenesis.
- 75. (previously presented) The method of claim 65, wherein the modifications are introduced by exponential ensemble mutagenesis.
- 76. (previously presented) The method of claim 65, wherein the modifications are introduced by site-specific mutagenesis.

じ

Attorney's Docket No.: 09010-027003 / DIVER1350-2

Applicant: Walter Callen et al. Serial No.: 09/656,309

Filed: September 6, 2000

Page : 7 of 19

77. (currently amended) A method of generating a <u>nucleic acid encoding variant</u> that encodes a polypeptide having <u>a polymerase activity comprising</u>:

obtaining a nucleic acid comprising a fragment of at least 30 consecutive nucleotides of a sequence as set forth in SEQ ID NO:1 and encoding a polypeptide having <u>a</u> polymerase activity or its complement; and

modifying one or more nucleotides in said sequence to another nucleotide, deleting one or more nucleotides in said sequence, or adding one or more nucleotides to said sequence, to generate a variant <u>nucleic acid</u> that encodes a polypeptide having polymerase activity; and

screening the polypeptide for a polymerase activity, thereby generating a polypeptide having a polymerase activity.

- 78. (previously presented) The method of claim 77, wherein the modifications are introduced by a method selected from the group consisting of error-prone PCR, shuffling, oligonucleotide-directed mutagenesis, assembly PCR, sexual PCR mutagenesis, in vivo mutagenesis, cassette mutagenesis, recursive ensemble mutagenesis, exponential ensemble mutagenesis, site-specific mutagenesis, ligation reassembly, gene site saturated mutagenesis (GSSM) and any combination thereof.
- 79. (previously presented) The method of claim 77, wherein the modifications are introduced by error-prone PCR.
- 80. (previously presented) The method of claim 77, wherein the modifications are introduced by shuffling.
- 81. (previously presented) The method of claim 77, wherein the modifications are introduced by oligonucleotide-directed mutagenesis.
- 82. (previously presented) The method of claim 77, wherein the modifications are introduced by assembly PCR.

Applicant: Walter Callen et al.

Serial No.: 09/656,309 Filed: September 6, 2000

Page : 8 of 19

83. (previously presented) The method of claim 77, wherein the modifications are introduced by sexual PCR mutagenesis.

84. (previously presented) The method of claim 77, wherein the modifications are introduced by in vivo mutagenesis.

85. (previously presented) The method of claim 77, wherein the modifications are introduced by cassette mutagenesis.

86. (previously presented) The method of claim 77, wherein the modifications are introduced by recursive ensemble mutagenesis.

87. (previously presented) The method of claim 77, wherein the modifications are introduced by exponential ensemble mutagenesis.

88. (previously presented) The method of claim 77, wherein the modifications are introduced by site-specific mutagenesis.

89 (new): The method of claim 31 or claim 65, wherein the polymerase activity is a thermostable polymerase activity.

90 (new): The method of claim 89, wherein the thermostable polymerase activity comprises activity at a temperature in a range from about 95°C to 113°C.

91 (new): The method of claim 31 or claim 65, wherein the polymerase activity comprises a $3' \rightarrow 5'$ exonuclease activity.

92 (new): The method of claim 31 or claim 65, wherein the polymerase activity comprises an activity that can function under conditions of high salinity.

Attorney's Docket No.: 09010-027003 / DIVER1350-2

Applicant: Walter Callen et al.

Serial No.: 09/656,309

Filed: September 6, 2000

Page : 9 of 19

93 (new): The method of claim 31 or claim 65, wherein the nucleic acid encoding the polymerase has a high guanidine-cytosine (GC) content.

94 (new): The method of claim 31 or claim 65, wherein the polymerase activity comprises amplifying a template sequence during PCR amplification procedures.

95 (new): The method of claim 31 or claim 65, comprising obtaining a nucleic acid comprising a sequence having at least 80% sequence identity to the sequence set forth in SEQ ID NO:1 and encoding a polypeptide having a polymerase activity.

96 (new): The method of claim 95, comprising obtaining a nucleic acid comprising a sequence having at least 90% sequence identity to the sequence set forth in SEQ ID NO:1 and encoding a polypeptide having a polymerase activity.

97 (new): The method of claim 96, comprising obtaining a nucleic acid comprising a sequence having at least 95% sequence identity to the sequence set forth in SEQ ID NO:1 and encoding a polypeptide having a polymerase activity.